

139. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain TCRV γ /VL.
- 5 140. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain TCRV δ /VL.
- 10 141. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain TCRV γ /VH.
- 15 142. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain TCRV δ /VH.
- 20 143. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain VL/TCRV γ .
144. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain VL/TCRV δ .
- 25 145. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain VH/TCRV γ .
- 30 146. The vector of claim 130, wherein the vector comprises a polynucleotide encoding a single chain VH/TCRV δ .

147. The vector of claim 130, wherein the polynucleotide encoding the T-cell receptor (TCR) and the immunoglobulin elements, fragments, domains and/or segments are in a tail-to-head transcriptional
5 orientation.

148. The vector of claim 130, wherein the vector is selected from the group consisting of plasmids, phages, phagemids, viral vectors and combinations thereof.

10

149. The vector of claim 130, further comprising transcription and translation control sequences.

15

150. The vector of claim 130, wherein the transcription control sequence is selected from the group consisting of a promoter, an RNA polymerase initiation site, an RNA polymerase termination site, a TATA box, a CAT box, a poly A addition site, an enhancer and a part or combination thereof.

20

151. The vector of claim 130, wherein the translation control sequence is selected from the group consisting of a ribosome binding site, a leader sequence and a part or combination thereof.

25

152. The vector of claim 130, wherein the vector is selected from the group consisting of pNMV, pCDV, p1PP12, PUC19-CK-CH1, PUM 19-CK-CHI and pJS.

30

153. An oligonucleotide comprising a nucleic acid having the sequence as set forth in Figure 1.

154. A method for creating a phage display chimeric T-cell receptor/immunoglobulin reagent comprising the steps of:

- (i) obtaining a sample of cells;
- 5 (ii) preparing mRNA
- (iii) reverse transcribing mRNA of the cell population into cDNA sequences of T-cell receptor and immunoglobulin;
- (iv) amplifying the cDNA;
- 10 (v) providing nucleic acid expression vectors which are capable of being packaged;
- (vi) cloning the population of DNA fragments into expression vectors;
- (vii) combining (i) a genetically diverse repertoire of nucleic acid sequences in which each encode a unique or genetically diverse population first component part of the TCR-cell receptor elements with (ii) a genetically diverse repertoire of nucleic acid sequences which encodes a unique or genetically diverse population of the immunoglobulin elements, to form a library of nucleic acid sequences using said expression vectors encoding said TCR and antibody polypeptide; also with the property of binding specifically to a target molecule of interest;
- 15
- 20
- 25
- (viii) expressing said library from said vectors in recombinant host organism cells, each of the said polypeptide chain components being expressed as a recombinant chimeric protein on its own or or as part of phage particles which are components of the library;
- 30
- (ix) selecting from said expressed library, by binding to a target molecule of interest, for

5 example with a MHC-peptide complex, a unique or restricted population of said reagents binding specificity, thereby producing a recombinant chimeric T-cell receptor/immunoglobulin reagent.

155. A method for creating a phage display T-cell receptor reagent comprising the steps of:

- (i) obtaining a sample of cells;
- 10 (ii) preparing mRNA
- (iii) reverse transcribing mRNA of the cell population into cDNA sequences of T-cell receptor;
- (iv) amplifying the cDNA;
- 15 (v) providing nucleic acid expression vectors which are capable of being packaged;
- (vi) cloning the population of DNA fragments into expression vectors;
- 20 (vii) combining a genetically diverse repertoire of nucleic acid sequences in which each encode a unique or genetically diverse population first component part of the TCR-cell receptor elements, to form a library of nucleic acid sequences using said
- 25 expression vectors encoding said TCR polypeptide; also with the property of binding specifically to a target molecule of interest;
- 30 (viii) expressing said library from said vectors in recombinant host organism cells, said polypeptide chain components being expressed as a recombinant TCR protein on its own or as part of phage particles which are components of the library;

- 5 (ix) selecting from said expressed library, by binding to a target molecule of interest, for example with a MHC-peptide, a unique or restricted population of said reagents binding specificity, thereby producing a recombinant T-cell receptor reagent.

10 156. A primer comprising the nucleic acid having the sequence as set forth in Figure 6, 7.

157. A method for selection against a target molecule, said method comprising:

- 15 i) contacting the library of claims 1 or 45 with the target molecule so as to form a complex,
ii) dissociating the bound phage from the complex;
iii) amplifying bound phage by growth in bacterial host;
20 iv) repeating round of binding, dissociation and amplification; and
v) screening said selected library on a target molecule.

25 158. The method of claim 157, further comprising characterizing the selected phage.

159. The method of claim 157, wherein the target and/or library are labeled.

30 160. The method of claim 157, wherein the library is attached to a target molecule bound to a support matrix.

161. The method of claim 160, wherein the support is a plastic dish, a virus particle, or a cell culture.

162. The method of claim 157, wherein the target molecule comprises cells such as tumor cells, viral infected cells, cells originated from tissue or organs.

163. A method for diagnosing a subject with a tumor, comprising the steps of:

- 10 i) Obtaining a sample from the subject,
- ii) Contacting the sample with a recombinant reagent of claims 72-128, wherein the reagent is specific for a specific tumor antigen so as to form a complex,
- 15 iii) Detecting the complex, the presence of which is indicative of a subject having the tumor.

164. Use of a pharmaceutical composition according to any of claims 56-90 for the prevention or treatment of a disease selected from the group consisting of HLA class I associated diseases (ankylosing spondylitis, Reiter disease, psoriatic spondylitis, psoriasis vulgaris and Behcet disease) and rheumatoid arthritis, pauciarticular juvenile rheumatoid arthritis, systemic lupus erythematosus, Sj6gren disease, IDDM, Addison disease, Graves disease, Hashimoto disease, celiac disease, primary biliary cirrhosis, pemphigus vulgaris, epidermolysis bullosa acquisita, Hodgkin disease, cervical squamous cell carcinoma, multiple sclerosis, optic neuritis, narcolepsi, myasthenia gravis, Goodpasture syndrome and alopecia areata).

165. A method of treating a subject with a disease or a pathogenic conditions, comprising administrating to

the subject an effective amount of the reagent of claims 74-130, thereby treating the subject with the disease or pathogenic condition.

- 5 166. A method for imaging a neoplastic disorder in a subject comprising the steps of administering to the subject an amount of the recombinant reagent of claims 74-130, wherein the reagent is labeled, and detecting the label.

10

167. A method of purifying and detecting the reagent of the recombinant phage library of claim 1, wherein the reagent comprises a linker region.

- 15 168. A method of purifying and detecting the reagent of the recombinant phage library of claim 1, wherein the reagent comprises a tag.

20

25

30

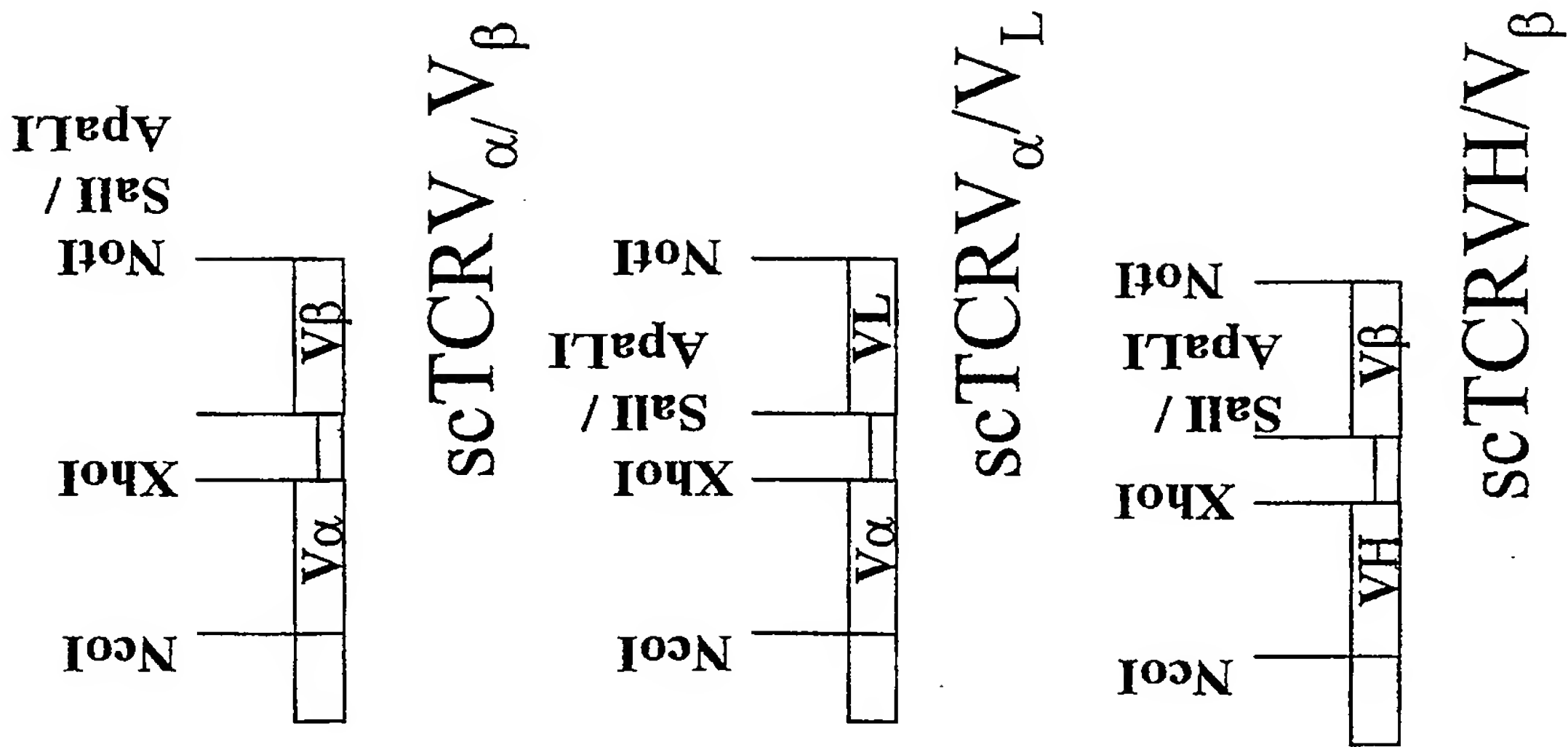


FIG .1B

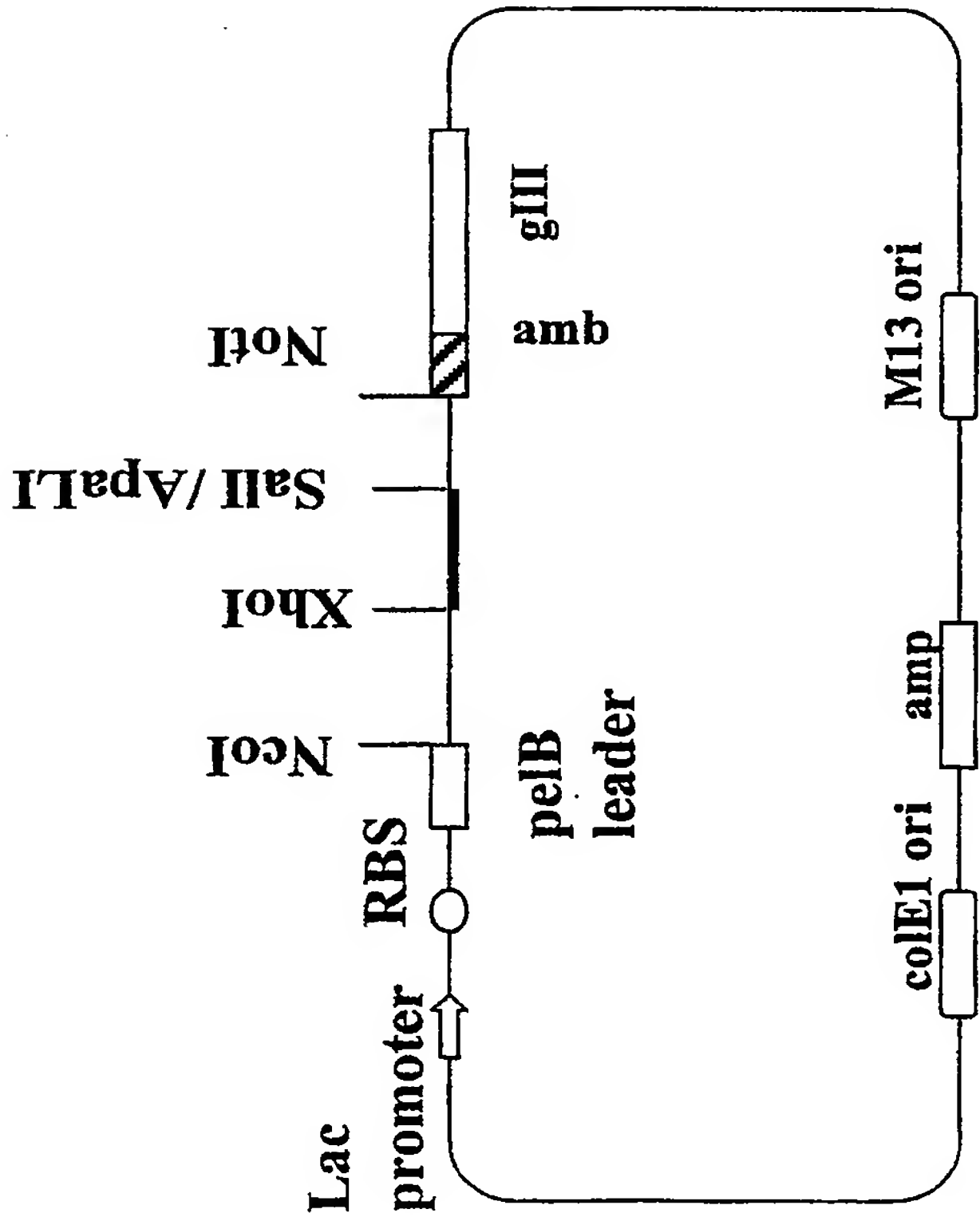
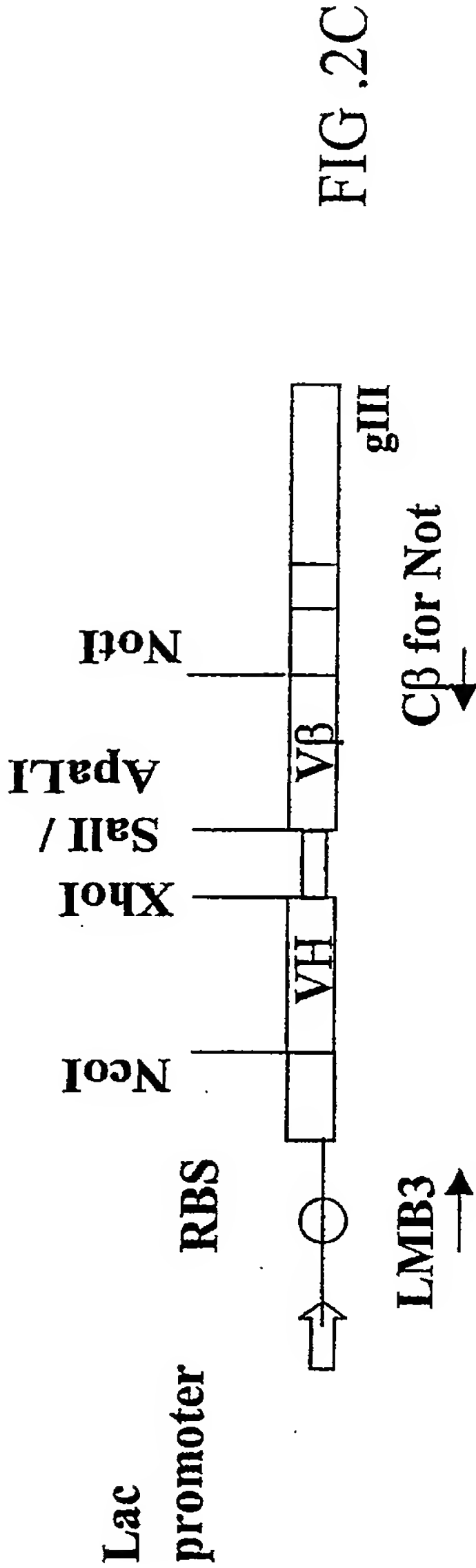
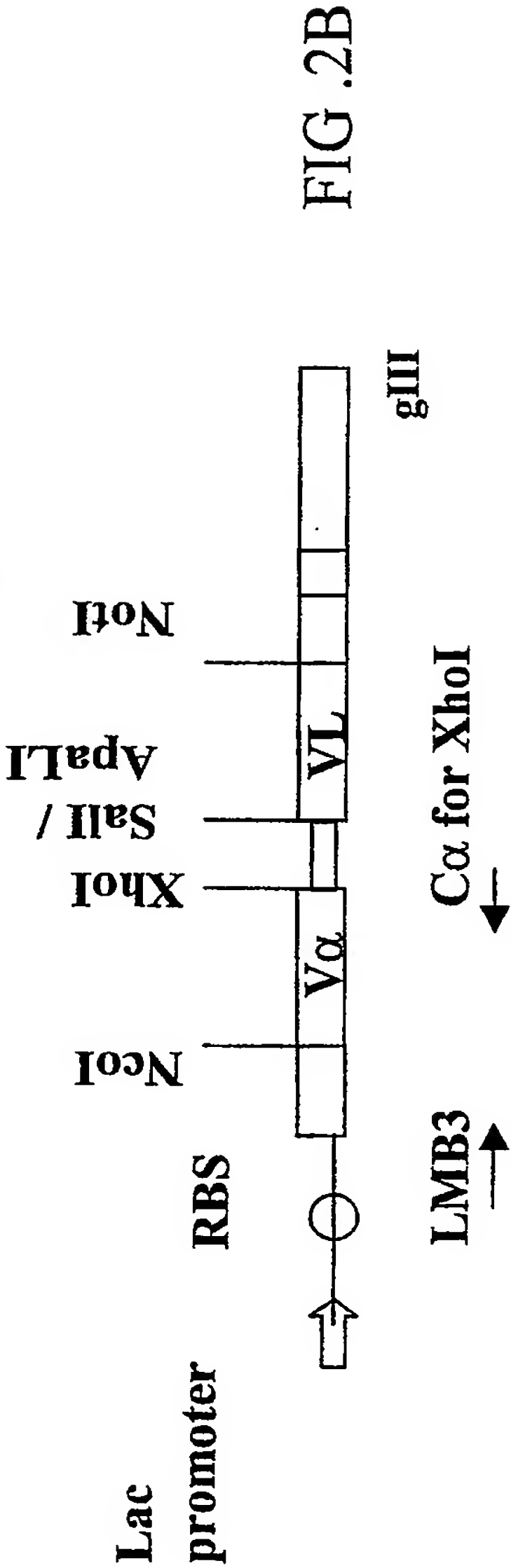
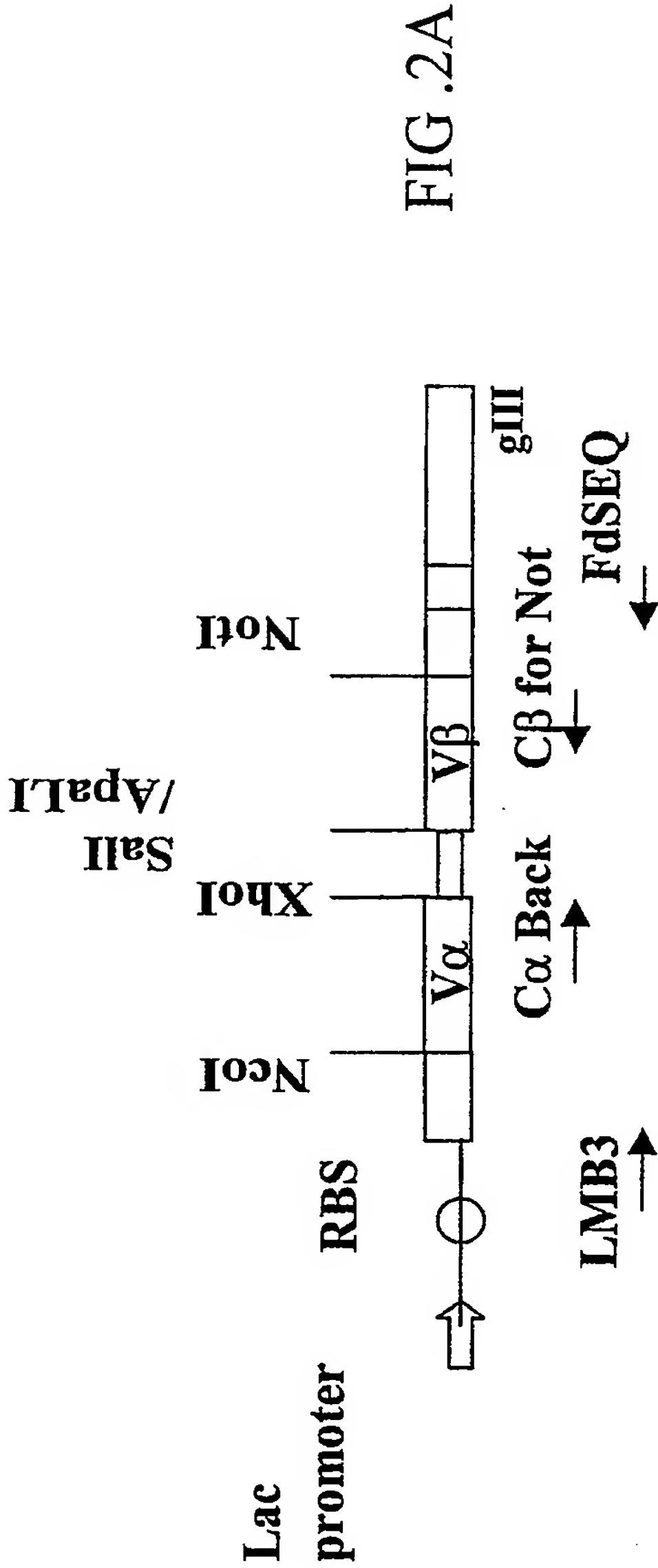


FIG .1A



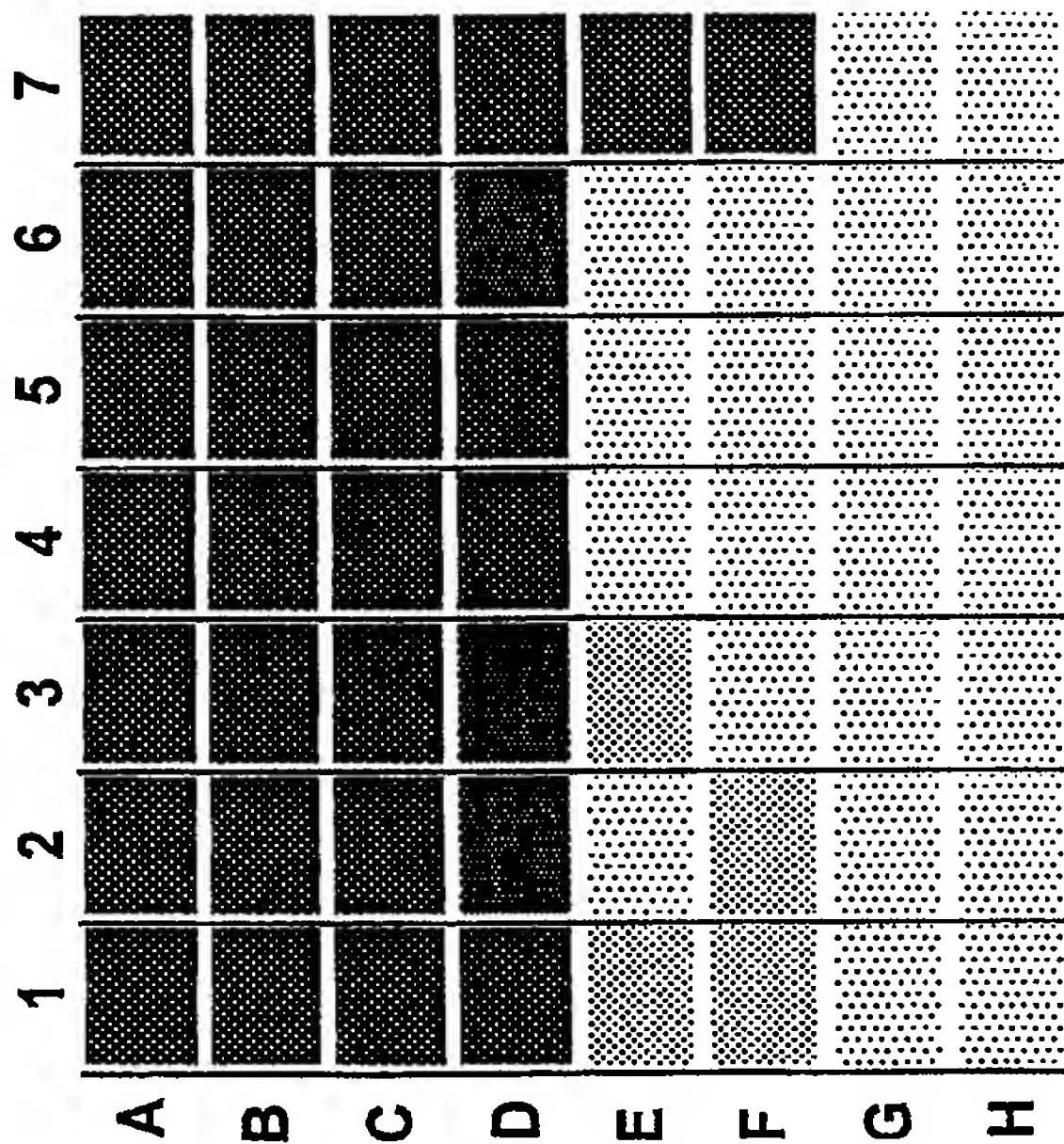


FIG.3B

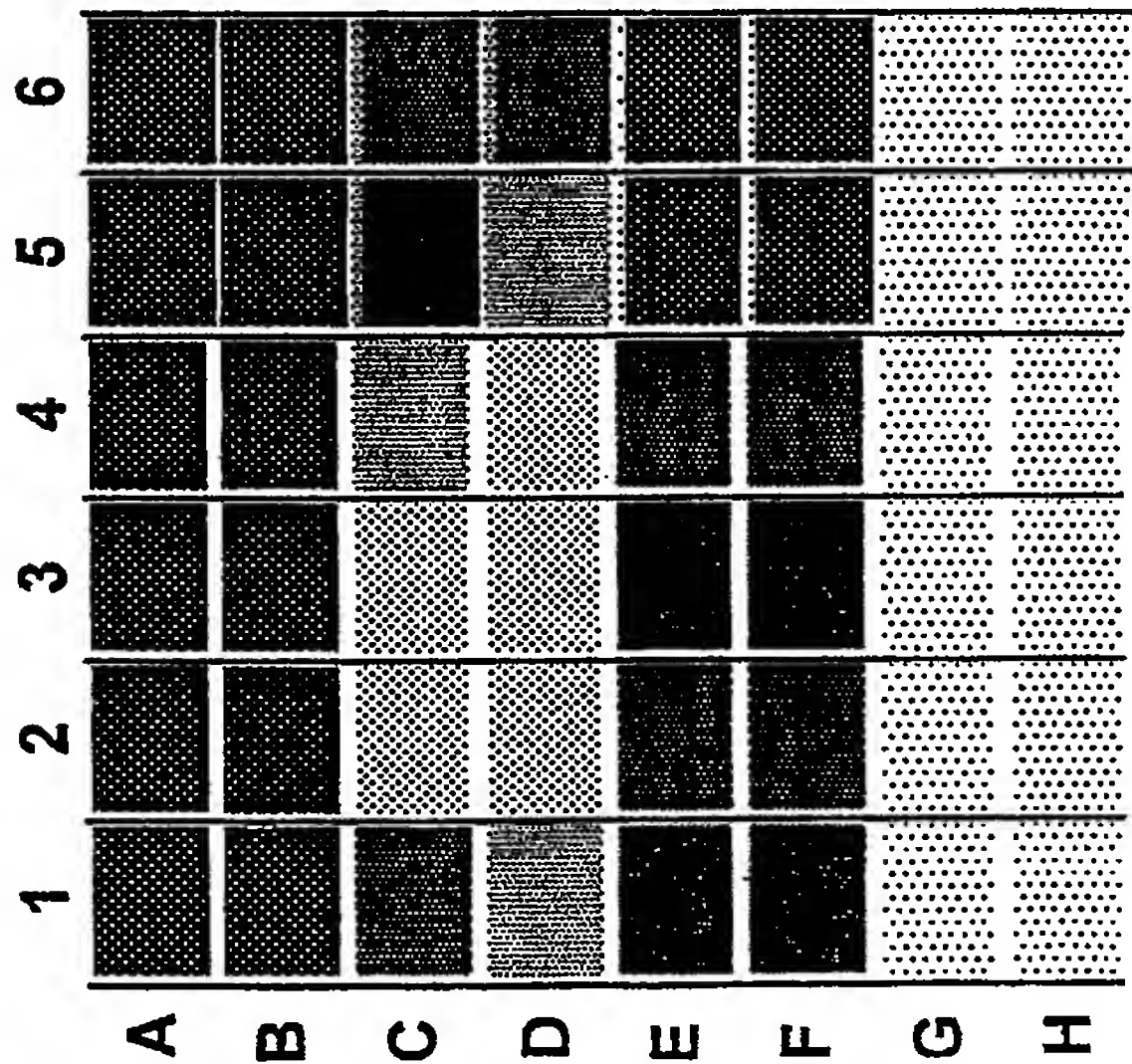


FIG.3A

ScTCRV α /VL	ScTCRVH/V β
Protein A/L	Protein A/L
0.055/1.078	0.550/0.012
0.043/0.431	0.319/0.011
0.078/1.486	0.266/0.010
0.074/0.249	0.168/0.009

FIG.3C

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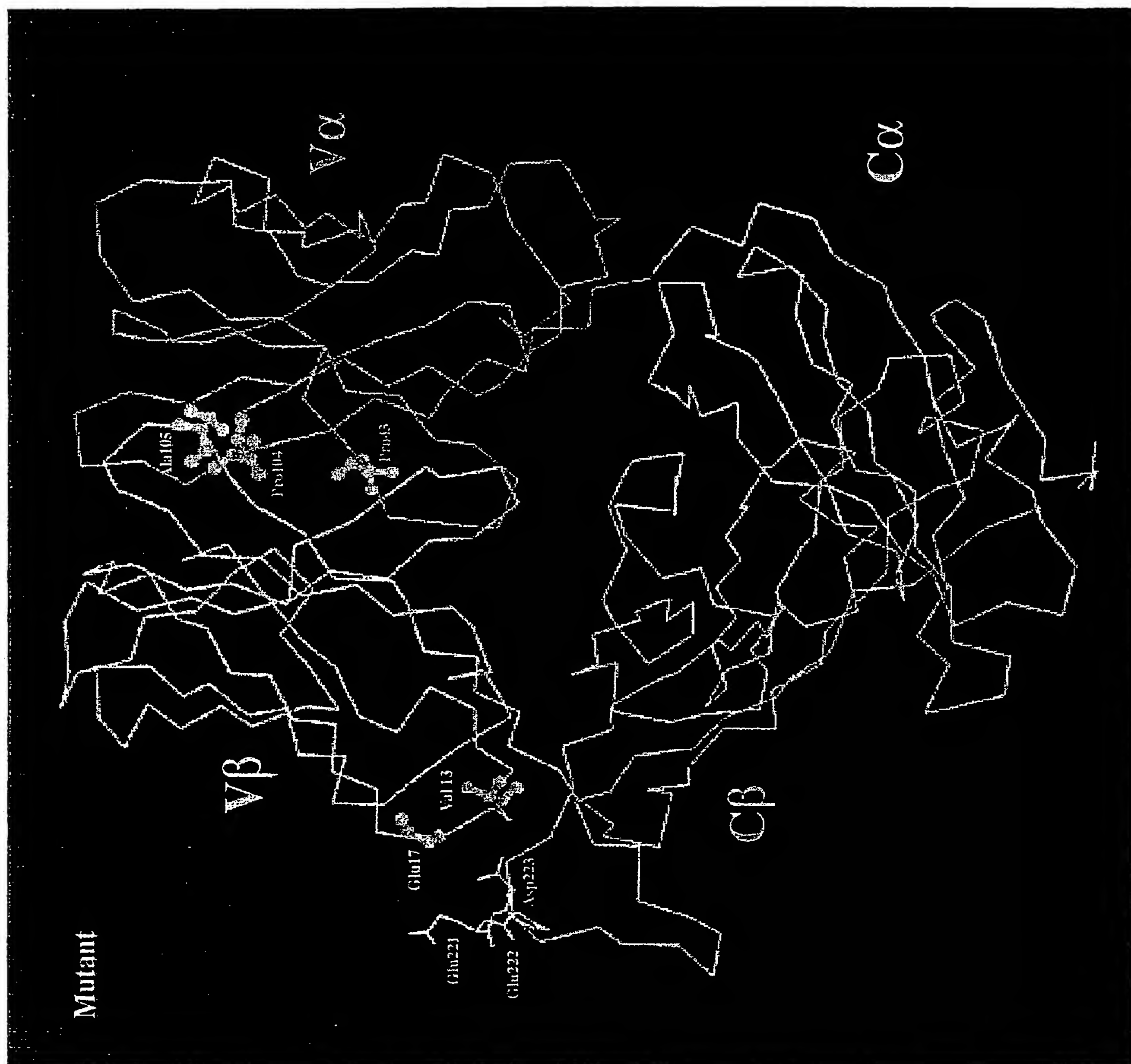


FIG.4A

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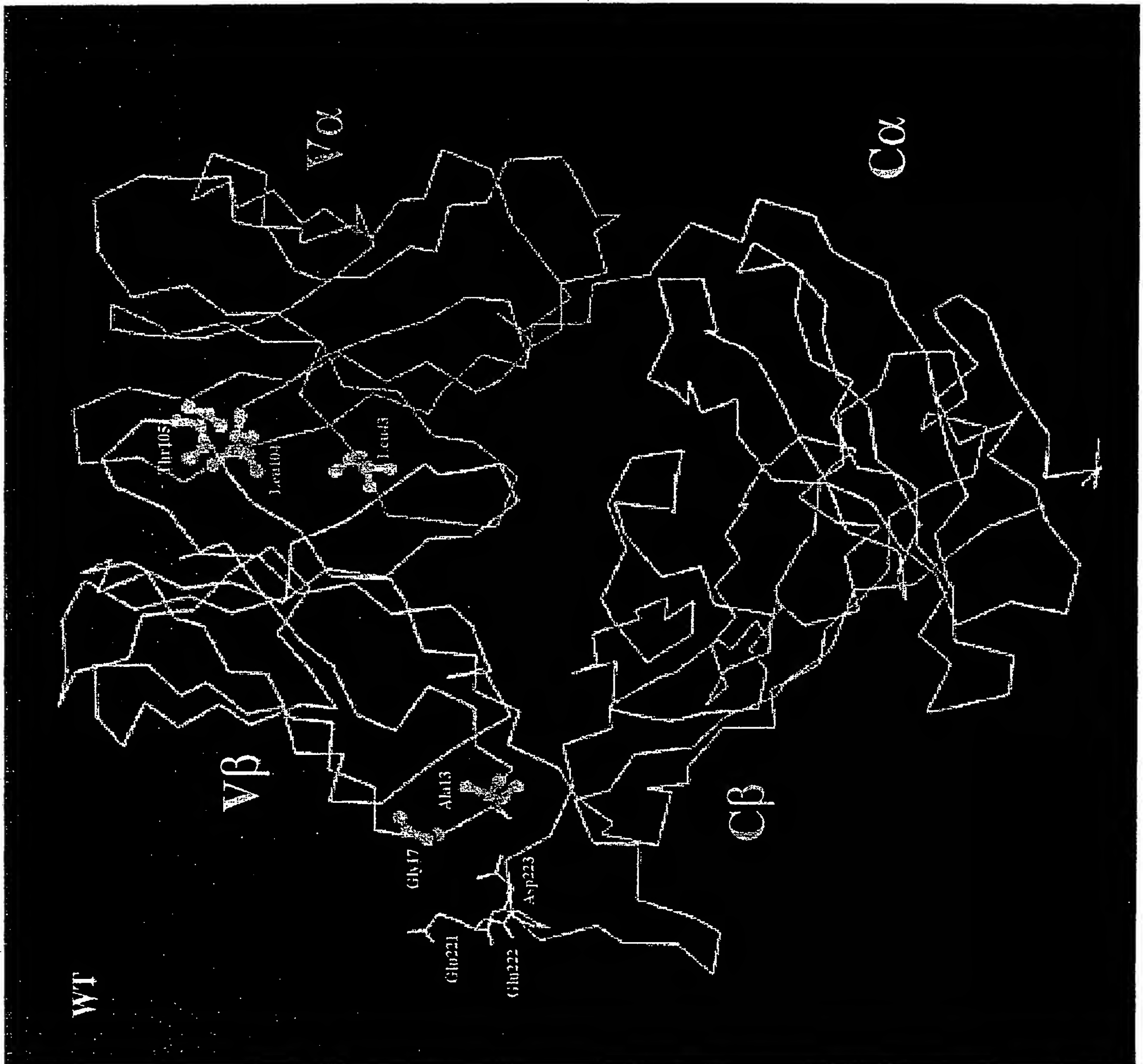


FIG.4B

V α -----GAC TCT AAA TCC AGT GAC AAG TCT GTC TCG AGC GGT GGA GGC GGT TCA GGC GGA GGT GGC AGC GGC
D S K S S D K S V S S G G G S G G G S G

SalI NotI
AGC GGC GGT GGC GGG TCG ACG -----GAG GAC CTG AAC AAG GTG GCG GCC GCA
G G G S T E D L N K V A A A

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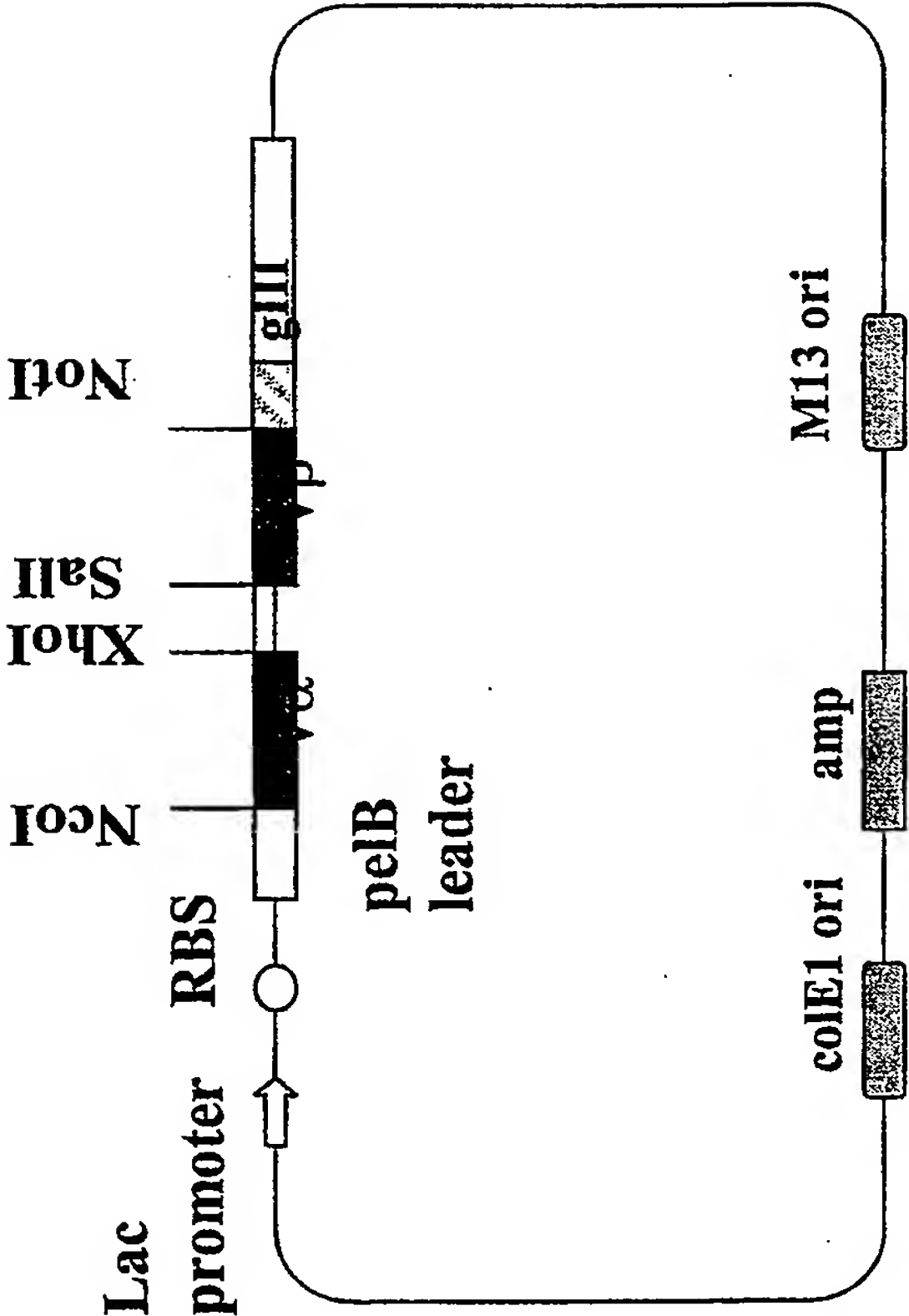


FIG. 5B

Vα-gene	Oligo
AV1S1 (AVS2-5)	GCG GCC CAG CCG GCC ATG GCC CAG TCK GTG ASC CAG CWT
AV2S1 (AV2S2-3)	GCG GCC CAG CCG GCC ATG GCC CAG GAG CTG GAG CAG RAT
AV3S1	GCG GCC CAG CCG GCC ATG GCC CAA CAG GGA GAA GAG GAT
AV4S1-2, AV20S1	GCG GCC CAG CCG GCC ATG GCC GCT AAG ACC ACC CAG CCC
AV6S1	GCG GCC CAG CCG GCC ATG GCC CAG AAG ATA ACT CAA ACC
AV7S1 (AV7S2)	GCG GCC CAG CCG GCC ATG GCC CAA ARC MTT GAS CAG CCC
AV8S1	GCG GCC CAG CCG GCC ATG GCC GAG ART GTG GRG CWG CAT
AV9S1	GCG GCC CAG CCG GCC ATG GCC CAG AGA GTG ACT CAG CCC
AV10S1	GCG GCC CAG CCG GCC ATG GCC CAG CTG CTG GAG CAG AGC
AV11S1	GCG GCC CAG CCG GCC ATG GCC GAC CAA GTG TTT CAG CCT
AV12S1	GCG GCC CAG CCG GCC ATG GCC CAG AAG GTA ACT CAA GCG
AV14S1-2	GCG GCC CAG CCG GCC ATG GCC CAG ACA GTC ACT CAG TCT
AV16S1	GCG GCC CAG CCG GCC ATG GCC CAG TCA GTG GCT CAG CCG
AV18S1 (AV15S1)	GCG GCC CAG CCG GCC ATG GCC GTG RAY GTG GAG CAA MGT
AV19S1 (AV24S1, AV13S1)	GCG GCC CAG CCG GCC ATG GCC AWH SAA GTG GAG CAG AGT
AV21S1 (AV5S1)	GCG GCC CAG CCG GCC ATG GCC CAG MAA RTT AAG CAA AAT

FIG. 6A

AV22S1A1 (AV22S1A2)	GCG GCC CAG CCG GCC ATG GCC	<u>RAT</u> TCA GTG ACC CAG ATG
AV23S1 (AV32S1)	GCG GCC CAG CCG GCC ATG GCC	CAR <u>SAG</u> GTR AYR CAR ATT
AV26S1 (AV25S1, AV17S1, AV30S1)	GCG GCC CAG CCG GCC ATG GCC	<u>SAV</u> <u>SAR</u> STG RMD CAR AGT
AV27S1	GCG GCC CAG CCG GCC ATG GCC	CTG AAA GTG GAA CAA AAC
AV28S1	GCG GCC CAG CCG GCC ATG GCC	GAC AAG GTG GTA CAA AGC
AV29S1	GCG GCC CAG CCG GCC ATG GCC	CAA CCA GTG CAG AGT CCT
AV31S1	GCG GCC CAG CCG GCC ATG GCC	AAT TCA GTC AAG CAG ACG
Cα-For- Xho	GTGCATAGCTGCTCGAGACAGACTTGTC	ACTGGATTAGAGTC

FIG. 6B

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Template TCRV β gene	Sequence
BV1S1A1N1 (BV11S1A1T, BV14S1, BV11S2OP)	CGC CGC AGT GTT AGG TCG ACG SGM RTS WMA CAA AMC CCA
BV2S1A2	CGC CGC AGT GTT AGG TCG ACG GTC GTC TCT CAA CAT CCG
BV3S1	CGC CGC AGT GTT AGG TCG ACG AAA GTA ACC CAG AGC TCG
BV4S1A1T	CGC CGC AGT GTT AGG TCG ACG GTC ATC TCT CAA AAG CCA
BV5S5P,6,3,7,2 (BV5S4)	CGC CGC AGT GTT AGG TCG ACG GGA GTC ACM CAA AGT CCC
BV6S4A1,3,5 (BV6S4A2, BV6S5A1N1, BV6S5A2, BV26S1, BV6S6, 3, BV22S1A2N1,2)	CGC CGC AGT GTT AGG TCG ACG GRA GTC WSC CAG DMY CCC
BV21S3A1,2 (BV21S1, BV16, BV6S5A1N2T) -	CGC CGC AGT GTT AGG TCG ACG GGR GTC TYY CAR TCY CCA
BV6S8,9 (BV23S1A1,2T, BV5S1A1T, BV5S1A2T)	CGC CGC AGT GTT AGG TCG ACG GGA GTC TCC CAG TCC CCT
BV6S2	CGC CGC AGT GTT AGG TCG ACG GGA GTC TCC CAG TCC CTG
BV6S7P	CGC CGC AGT GTT AGG TCG ACG GGA GTC TCC CAG TCC CTG
BV7S1A1N2T (BV27S1P, BV13S2A1T, S1)	CGC CGC AGT GTT AGG TCG ACG GRW RTY ACB CAG ACM CCA
BV7S2A2T (BV30S1A1, BV31S1P, BV13S5)	CGC CGC AGT GTT AGG TCG ACG GGR RTY ASG CAR RYA CCA
BV8S1-2 (BVS4, BVS5)	CGC CGC AGT GTT AGG TCG ACG GGH RTT ATC CAG TCA CCC
BV8S3& (BV19S1P,2)	CGC CGC AGT GTT AGG TCG ACG ARA GTC ACM CAG ACW CCA

FIG. 7A

BV9S1 (BV9S2)	CGC CGC AGT GTT AGG TCG ACG	GCT GTT TCC CAG ACT CCA
BV10S1P	CGC CGC AGT GTT AGG TCG ACG	AAG GTC ACC CAG AGA CCT
BV12S1A1N1 (BV12S2A1T, BV17S1A3T, BV29S1P)	CGC CGC AGT GTT AGG TCG ACG	GRM ATC WMY CAG WBS CCA
BV15S1	CGC CGC AGT GTT AGG TCG ACG	GAT GTT ACC CAG ACC CCA
BV20S1A	CGC CGC AGT GTT AGG TCG ACG	ACT ATT CAT CAA TGG CCA
BV21S2A1,2	CGC CGC AGT GTT AGG TCG ACG	GGA GTG GTT CAG TCT CCC
BV21S3A1,2 (BV21S1, BV16, BV6S5A1N2T)	CGC CGC AGT GTT AGG TCG ACG	GRA GTT DCC CAG TYT CCC
BV24S1A2T (BV18S1)	CGC CGC AGT GTT AGG TCG ACG	RKS GTC ATG CAG AAC CCA
BV25S1A1,2,3	CGC CGC AGT GTT AGG TCG ACG	GAA GAA GTC GCC CAG ACT
BV28S1P	CGC CGC AGT GTT AGG TCG ACG	GTA GTT ACA CAA TTC CCA
BV32S1P	CGC CGC AGT GTT AGG TCG ACG	GGG ATC ACC CAG ATG CCA
BV33S1P	CGC CGC AGT GTT AGG TCG ACG	GAA GCC ACC TAG ACT CTA
BV34S1P	CGC CGC AGT GTT AGG TCG ACG	AAA GTA ACA CAG ACC
Cβ1-F0R-Not	GAGCCCATGATGTGCGGCGCCACCTTGTTCAGGTCCTC	
Cβ2-F0R-Not	CAGTGACCCATGTGCGGCGCCACGTTTTTCAGGTCCTC	

FIG. 7B

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V α GENE	J α GENE	V α GENE	J α GENE
AV28S1A3T	Tra42	AV22S1A2N1	TRA47
AV28S1A3T	TRA36	AV22S1A2N1	TRA4
AV28S1A3T	TRA37	AV22S1A2N1	Tra53
AV28S1A1T	TRA41		
AV28S1A3T	TRA26	AV1S4A1N1T	TRA44
AV28S1A1T	Tra43		
AV28S1A3T	TRA58	AV4S2A2T	TRA34
		AV4S2A2T	TRA49
AV6S1A1N1	Tra9		
AV6S1A2N2	Tra8	AV16S1A2PT	TRA13
AV6S1A2N2	Tra30		
AV6S1A1N1	Tra37	AV23S1	TRA40
AV6S1A2N1	Tra13	AV23S1	TRA16
AV6S1A2N2	Tra48	AV23S1	Tra9
AV6S1A2N2	Tra52		
AV6S1A1N1	Tra5	AV14S2A2N1	TRA10
AV6S1A2N2	TRA10	AV14S1	TRA50
AV6S1A1N1	TRA26	AV14S2A1T	TRA50
		AV14S2A1T	TRA50
AV21S1A1N	TRA6	AV14S2A1T	TRA50
AV21S1A1N1	TRA57	AV14S1	TRA50
AV21S1A1N1	TRA52		
AV21S1A1N	TRA36	AV20S1	TRA4
AV21S1A1N	TRA36	AV10S1A2	TRA49
		AV9S1	TRA9
AV27S1	TRA43		
AV27S1	TRA54	AV19S1	Tra 22
AV27S1	TRA31		
AV27S1	Tra50	AV19S1	Tra 54
AV27S1	Tra33	AV23S1	Tra 58
AV27S1	Tra56	AV23S1	Tra 21
AV27S1	TRA20		
AV27S1	TRA20		
AV27S1	TRA48		
AV27S1	TRA45		
AV27S1	TRA41		

FIG. 8A

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TCRV β 1 gene	J β gene	TCRV β 2 gene	TCR-J β
BV2S1A1	TRBJ1_1	BV2S1A1	2-7
BV2S1A1	trbj1-4	BV2S1A1	2-2
		BV2S1A1	2-3
BV3S1	TRB1-5	BV2S1A1	2-4
BV3S1	TRB1-4	BV3S1	2-7
		BV3S1	2-5
BV4S1A1T	TRB1-5	BV3S1	2-4
BV4S1A1T	TRB1-1	BV3S1	2-1
BV4S1A3T	TRB1-2	BV4S1A1T	2-5
		BV4S1A1T	2-3
BV5S4A2T	TRB1-1	BV4S1A1T	2-2
		BV4S1A1T	2-1
BV6S5A1N1	TRBJ 1-5	BV5S1A1T	2-7
BV6S5A1N1	TRBJ1_1	BV6S2A1N1T	2-5
		BV6S2A1N1T	2-3
BV8S2A1T	TRB1-1	BV6S4A1	2-1
BV8S1	TRB1-5	BV6S4A1	2-3
BV8S1	TRB1-2	BV6S4A1	2-7
		BV6S5A1N1	2-5
		BV6S5A1N1	2-3
BV9S1A1T	TRB1-5	BV7S1A1N2T	2-3
BV9A1A1T	TRBJ1_2 (172)	BV7S3A2T	2-7
		BV9S1A1T	2-7
		BV13S6A2T	2-2
BV13S1	TRBJ1_1	BV15S1	2-1
BV13S6A2T	TRBJ1_4	BV18S1	2-3
BV13S1	TRB1-1	BV19S1P	2-3
		BV19S1P	2-4
		BV19S1P	2-7
BV22S1A2N1	TRB1-5	BV21S2A2	2-2
		BV21S2A2	2-1
		BV22S1A2N1	1-1
		BV22S1A2N1	2-3

FIG. 8B

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TCRV α	J α
AV14S2A2N	TRA37
AV22S1A2N	TRA9
AV3S1	TRA47
ADV6S1A1N	NC
AV23S1	TRA22
ADV6S1A1N	TRA39
AV22S1A1N	TRA20
AV16S1A2P	TRA4
AV22S1A2N	TRA13

FIG. 9

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	TCR-V α	TCR-J α
Anti-IgER clones		
	AV21S1A1N	TRA57
	AV21S1A1N	TRA49
	AV21S1A1N	TRA30
	AV27S1	TRA20
	AV27S1	TRA27
	AV27S1	TRA27
	AV21S1A1N	TRA53
	AV6S1A2N2	TRA21
Anti-EGF clones		
	AV6S1A2N1	TRA5
	AV6S1A2N2	TRA22
	AV28S1A3T	TRA11
	AV6S1A2N2	TRA39
	AV16S1A2PT	TRA5
	AV22S1A2N1	TRA11
	AV27S1	TRA54
	AV8S1A1	TRA9
Anti-A431 clones	AV27S1	TRA57
	AV27S1	TRA49
	AV6S1A1N1	TRA49
	AV21S1A2PT	TRA49
	AV27S1	TRA52

FIG. 10

SEQUENCE LISTING

<110> NISSIM, Ahuva

<120> CHIMERIC AND TCR PHAGE DISPLAY LIBRARIES, CHIMERIC AND
TCR REAGENTS AND METHODS OF USE THEREOF

<130> 1312503- Ahuva NISSIM

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<170> PatentIn Ver. 2.1

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